Peer Review
Experimental Toxicology Division
NHEERL

PARTICULATE MATTER

Cardiopulmonary Effects of Acute and Subchronic Exposure to Concentrated Ambient Particulates in Healthy and Compromised Rats

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INTRODUCTION

- Epidemiological studies have consistently demonstrated small but significant correlations between the levels of ambient particulate matter (PM) and the incidence of cardiopulmonary-related morbidity and mortality; these correlations appear to be strengthened when limited to persons with pre-existing cardiopulmonary disease
- Numerous animal studies have been conducted to elucidate these issues, however, most have employed high concentrations of fairly toxic particles
- Relatively little is known regarding the effects of lower concentrations of more "environmentally-relevant" PM
- The goal of these studies was to examine the cardiac, pulmonary, and thermoregulatory effects of Concentrated Ambient Particulates (CAPs) in a number of rodent models of cardiopulmonary disease

METHODS

Experimental Conditions:

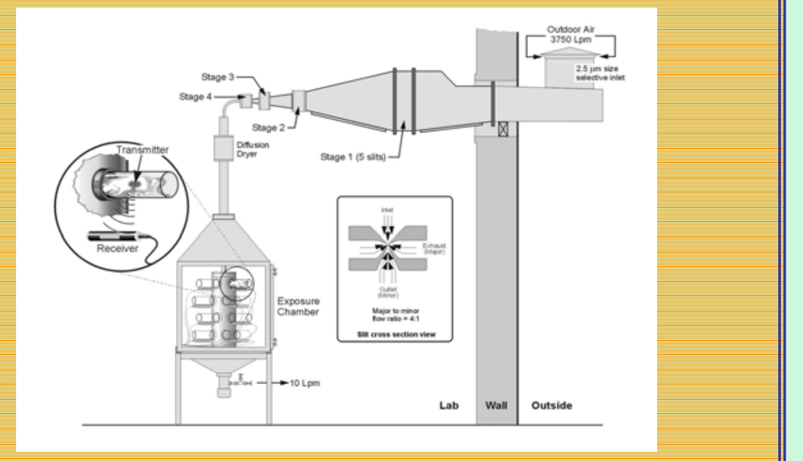
- animals: Spontaneously Hypertensive, Fischer-344 or Sprague-Dawley rats
- n=16–24/study; 4–12 rats/group
- experimental treatment: CAPs
- exposure regimens:
  1) 2–3 day/wk × 11 wk
  2) 1 day/wk × 11 wk
  3) 2–3 day/wk × 2 wk
  4) 2–3 day/wk × 1 wk
- exposure: nose-only inhalation
- sacrifice: 24 hours post-exposure

Procedure:

- implant radiotelemeters
- recovery period (10 d)
- control period (3 d)
- nose-only exposure
- monitor cardiopulmonary endpoints

Endpoints Assessed:

- heart rate, blood pressure, core temperature, QA interval
- activity
- body weight
- heart and lung weights
- ECG intervals/durations
- arrhythmias
- pulmonary function
- BALF constituents
- serum enzymes
- heart and lung histopathology

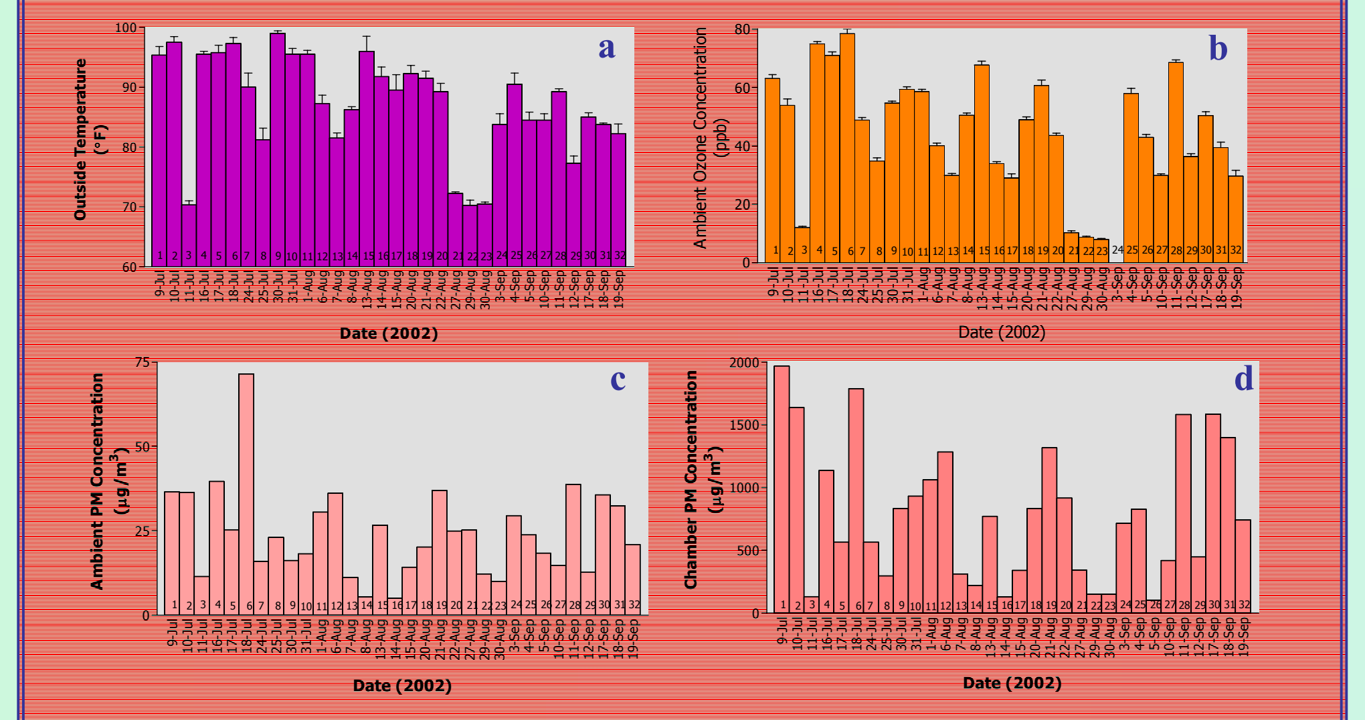


Concentrated Ambient Particulate Studies

INVESTIGATOR	YEAR	ANIMAL STRAIN/ DISEASE MODEL	EXPOSURE DURATION	PRIMARY ENDPOINTS	MOST IMPORTANT FINDINGS
Kodavanti	1997	Rat; SD bronchitis	2 d×5 reps	pulmonary inflammation pulmonary injury	↑ inflammation (3 of 5) ↑ BALF protein
Gavett	1998	Mouse; Balb/cJ, Wild Type, Mast Cell Deficient allergic asthma	2 d×3 reps	airway responsiveness pulmonary inflammation	WT: ↑ BALF eosinophils MCD: small ↑ BALF eosinophils
Gilmour	1999	Rat; SD healthy	3 d/wk×5 wk	pulmonary inflammation pulmonary injury	↑ BALF macrophages, neutrophils ↑ fibrinogen
Gilmour	1999	Mouse; CD1, C3H healthy	3 d×6 reps	pulmonary inflammation pulmonary bacteria	sporadic ↑ infectivity
Kodavanti	2000	Rat; SH systemic hypertension	2 d/wk×1 wk 2 d/wk×9 wks	BALF/blood biomarkers antioxidants	small ↑ fibrinogen
Kodavanti	2000	Rat; SH systemic hypertension	1 d×6 reps	cardiopulmonary function BALF/blood biomarkers	small ↑ fibrinogen
Dye	2001	Rat; SH, WKY Aged	2 d×1 rep	BALF/blood biomarkers antioxidants	age-related effects no CAPs potentiation of effects
Kodavanti	2001	Rat; SH, WKY systemic hypertension	2 d×9 reps	BALF/blood biomarkers antioxidants	WKY: ↑ neutrophils, ↓ total cells SH: ↑ fibrinogen, ↓ GGT
Costa	2002	Rat; SH systemic hypertension	1 d/wk×11 wks	cardiopulmonary function BALF/blood biomarkers	in progress
Dreher	2002	Rat; SD healthy	2–3 d/wk×11 wks	cardiac function	in progress
Dye	2002	Rat; SD antioxidant depleted	2 d×2 reps	BALF/blood biomarkers antioxidants cell proliferation	small ↑ neutrophils
Kodavanti	2002	Rat; WKY antioxidant depleted	2 d×2 reps	BALF/blood biomarkers antioxidant cell proliferation	small ↑ neutrophils
Rogers	2002	Rat; F-344 pregnant	3 d/wk×2 wks× 2 reps	maternal/fetal weight fetal morphology	in progress
Watkinson	2002	Rat; SH systemic hypertension	2-3 d×9 wks	cardiopulmonary function BAL/blood biomarkers	in progress
Watkinson	2002	Rat; SH systemic hypertension, aged	3 d/wk×1 wk	cardiopulmonary function BAL/blood biomarkers	in progress
Watkinson	2002	Rat; SD pulmonary hypertension	3 d/wk×1 wk	cardiopulmonary function BAL/blood biomarkers	in progress

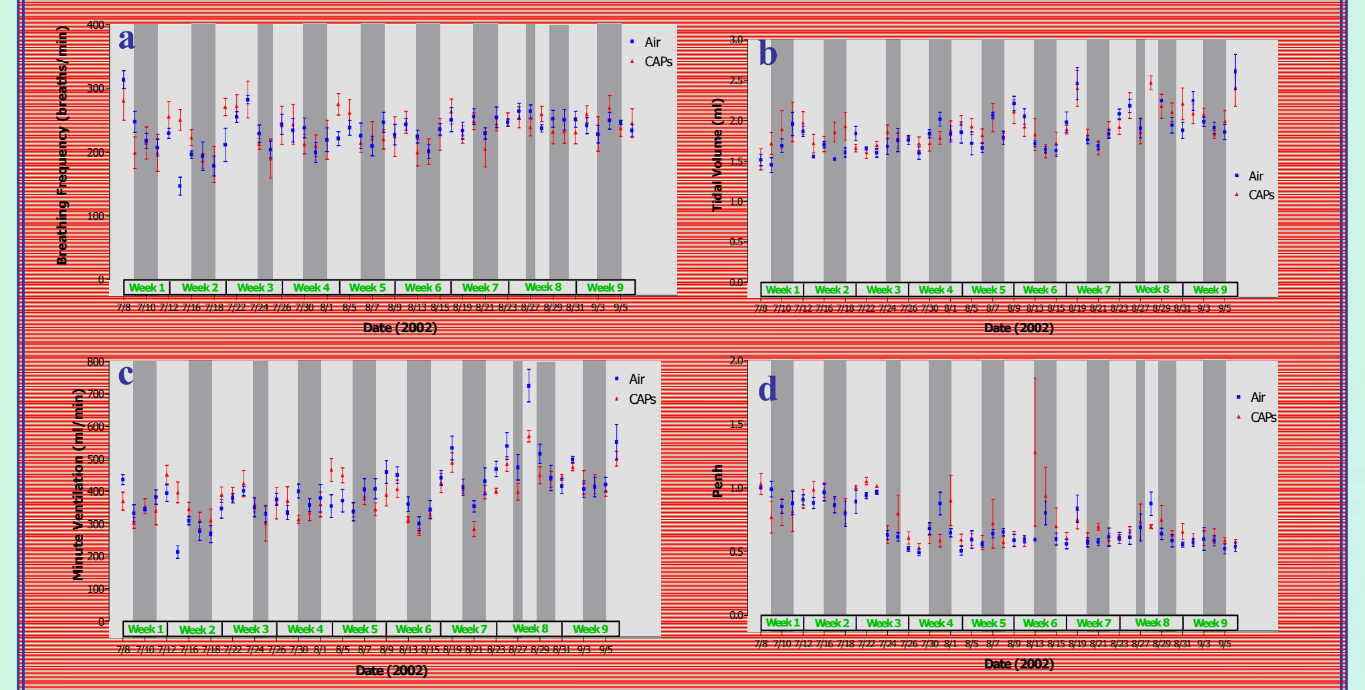
SUBCHRONIC RESULTS

Environmental Conditions



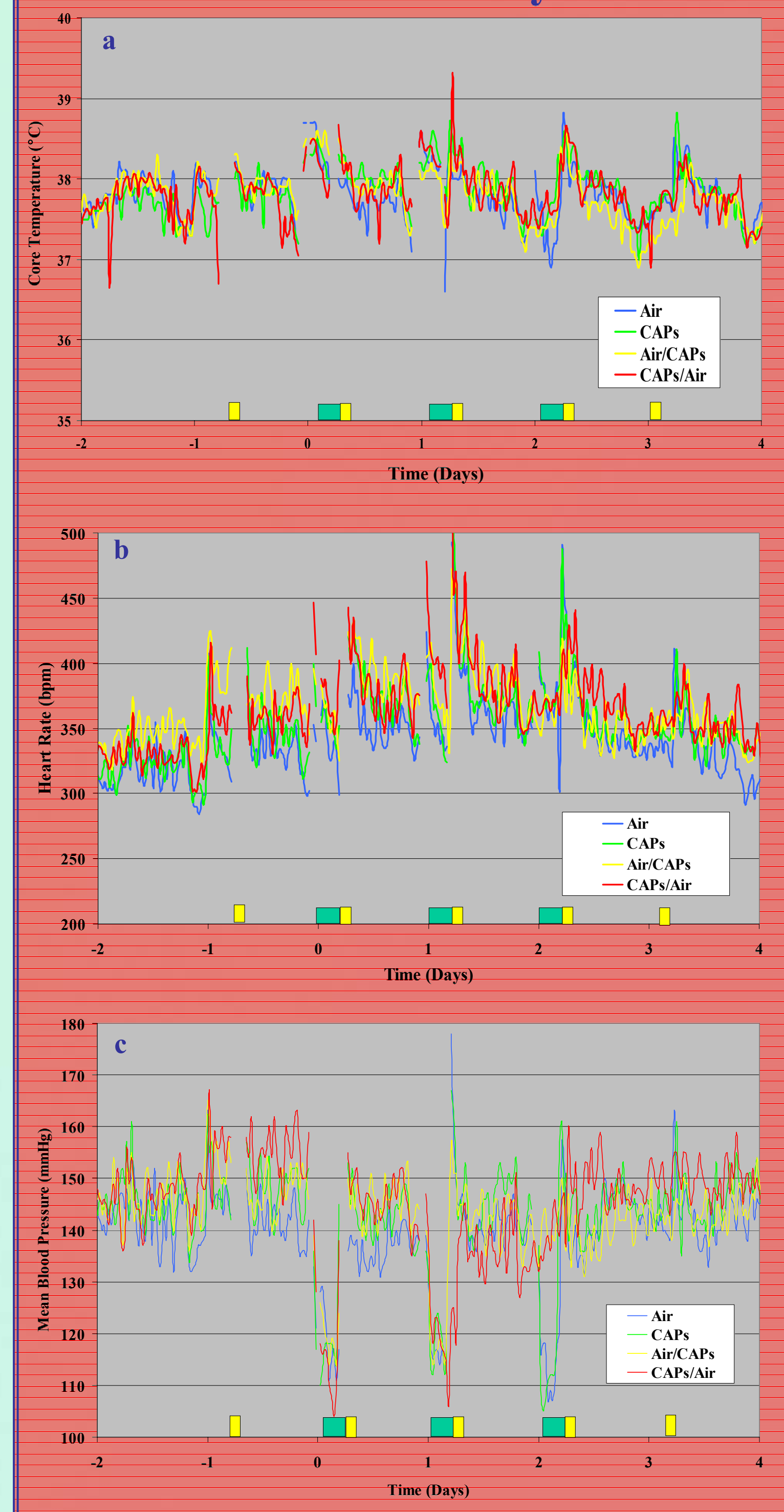
Environmental and Chamber Conditions During Experimental Time Period
Average daily values for ambient and chamber conditions during entire experimental time period (9 July–19 September). Charts show average values for (a) ambient temperature, (b) ambient ozone concentration, (c) ambient PM concentration, and (d) chamber PM concentration for each day that an exposure was conducted.

Whole-body Plethysmograph Data



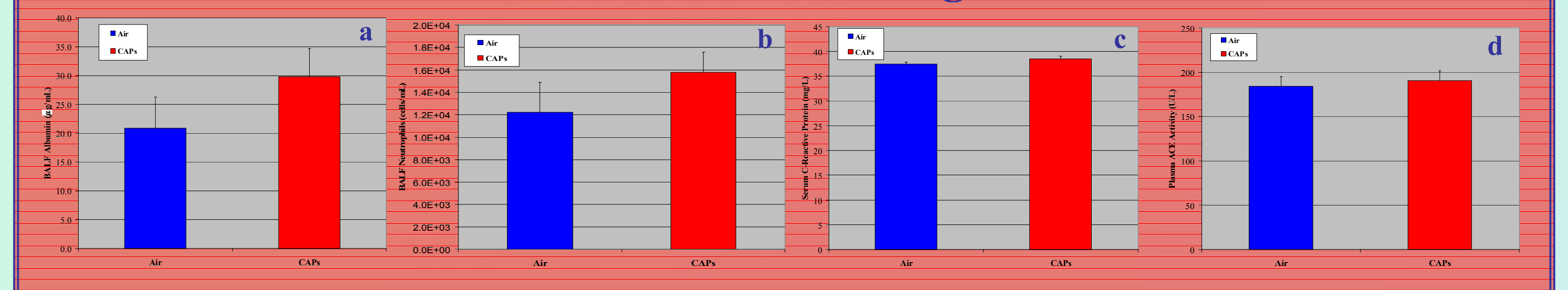
Changes in Pulmonary Function Parameters in a Rodent Model of Systemic Hypertension Following Exposure to Concentrated Ambient Particulates
Effects of nose-only exposure to concentrated ambient particles on (a) frequency of breathing, (b) tidal volume, (c) minute ventilation, and (d) penh of awake, adult Spontaneously Hypertensive rats (n=4/group). Exposures (4 hr/day × 2–3 days/week × 9 weeks) were conducted over the time period from 9 July–19 September 2002. Pulmonary function was evaluated using a Buxco whole-body plethysmograph system; evaluations were carried out on the day before each exposure, on the day of and immediately following each exposure (shaded bars), and on the day after each exposure. Values are shown as group means ± SE.

Telemetry Data



Changes in Core Temperature, Heart Rate, and Blood Pressure in a Rodent Model of Systemic Hypertension Following Exposure to Concentrated Ambient Particulates
Effects of nose-only exposure to concentrated ambient particles on (a) core temperature, (b) heart rate, and (c) blood pressure of awake, adult Spontaneously Hypertensive rats (n=4/group) during the first week of a nine-week exposure period. During exposure, animals were maintained in individual plexiglass tubes attached to a nose-only chamber that was located inside a climate-controlled enclosure. Exposures (4 hr/day × 2–3 days/week) were carried out beginning at ≈0930–1000 each day. When not undergoing exposure, animals were housed in individual plexiglass cages in a separate climate-controlled chamber. Electrocardiograms, heart rate, core temperature, and blood pressure were continually monitored using previously implanted radiotelemetry transmitters. Data were obtained at 5-min intervals but for clarity of presentation have been averaged across 30-min periods. Data are presented as group means. NOTE: Nose-only exposure periods are indicated by the green boxes above the abscissa; pulmonary function monitoring periods are indicated by the yellow boxes above the abscissa. The first exposure day is shown as Day=0.

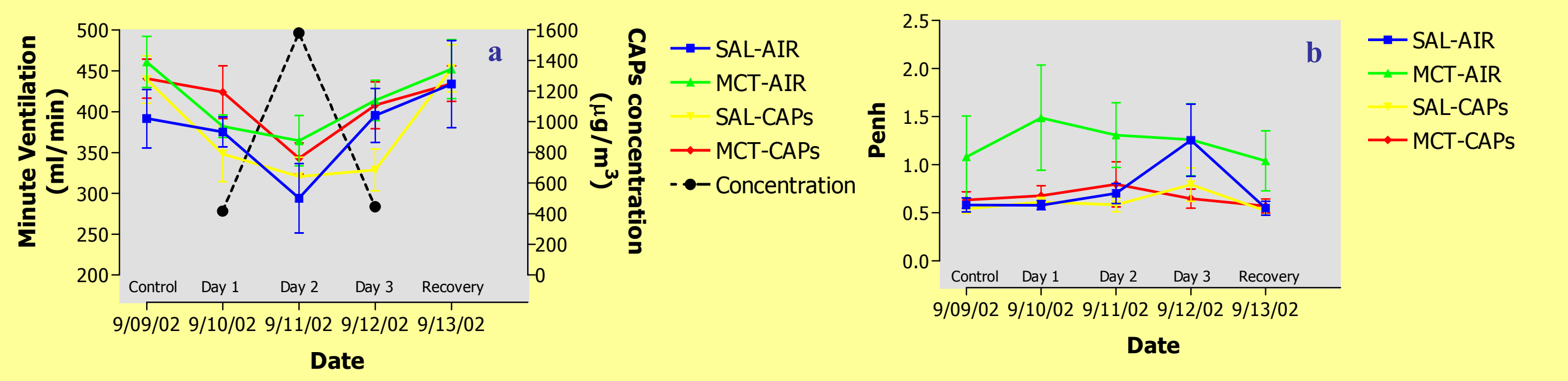
BALF and Hematologic Data



Changes in Bronchoalveolar Lavage and Hematologic Constituents in a Rodent Model of Systemic Hypertension Following Exposure to Concentrated Ambient Particulates
Effects of nose-only exposure to concentrated ambient particles on bronchoalveolar lavage and blood levels of (a) albumin, (b) neutrophils, (c) serum C-reactive protein, and (d) plasma ACE activity of awake, adult Spontaneously Hypertensive rats (n=4/group). Exposures (4 hr/day × 2–3 days/week × 9 weeks) were conducted over the time period from 9 July–19 September 2002. Bronchoalveolar lavage and blood samples were collected ≈24h following the final exposure. Values are shown as group means ± SE.

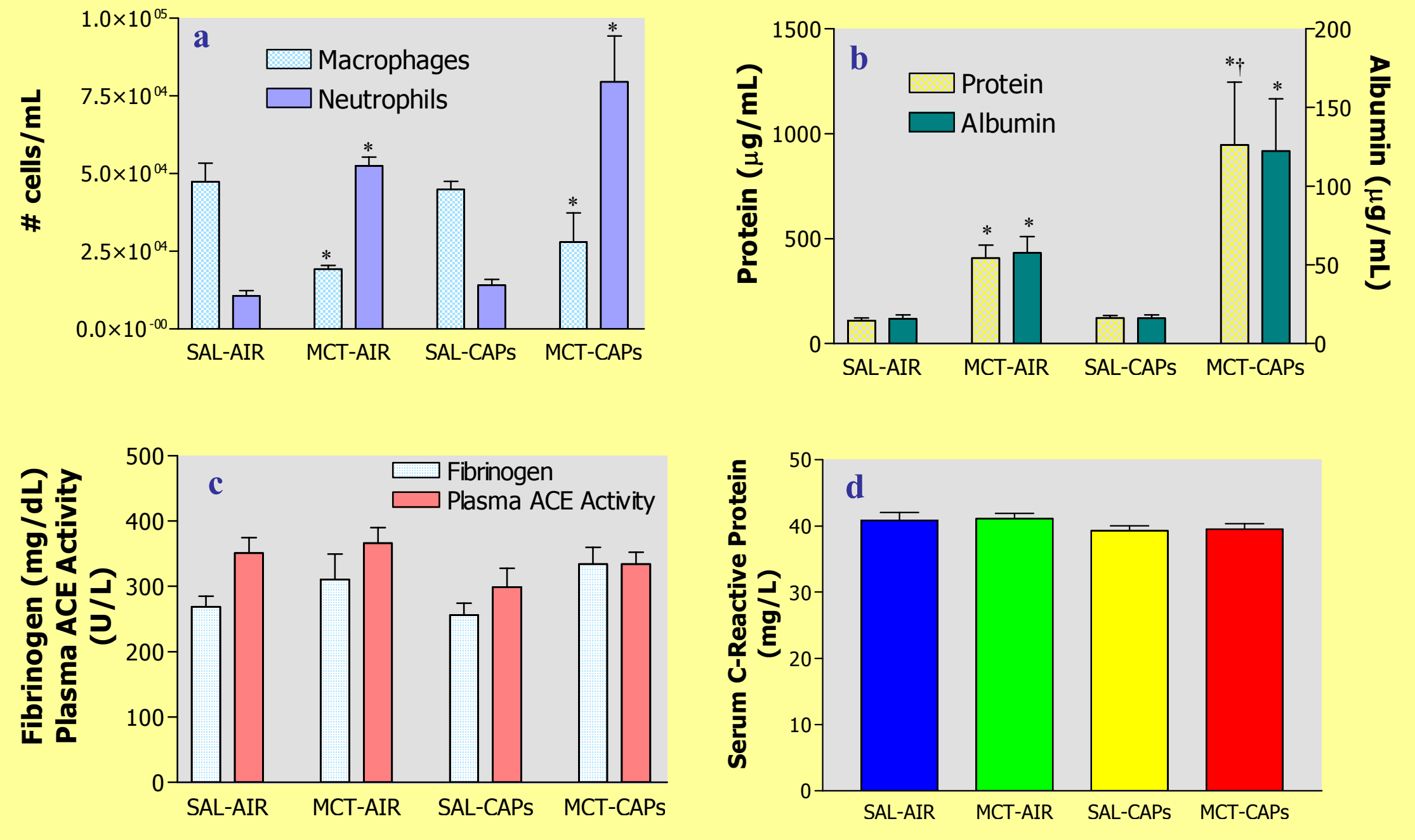
ACUTE RESULTS

Whole-body Plethysmograph Data



Changes in Minute Ventilation and Penh in a Rodent Model of Cardiopulmonary Disease Following Exposure to CAPs
Effects of nose-only inhalation exposure to RTP CAPs on (**a**) minute ventilation and (**b**) penh of awake, adult Sprague-Dawley rats (n=24 rats, 6 rats/group). A subset of rats was injected with monocrotaline and allowed to recover for 14 days prior to CAPs exposure. Respiratory parameters were monitored using a whole-body plethysmographic system (Buxco Electronics) for 10 min/d for 5 days. Data shown were collected one day prior to the first CAPs exposure (Control) and continued for one day post-exposure (Recovery); CAPs exposures occurred on September 10-12, 2002. The black line on **Figure a** represents the CAPs concentration in the exposure chamber for animals on that day (as shown on the right y-axis). Individual animal data were obtained at 10-s intervals but have been averaged over a 10-min period. Data in the figures are presented as group means ± SE; no values were found to be significantly different in either figure.

BALF and Hematologic Data



Acute Changes in Bronchoalveolar Lavage and Hematologic Constituents in a Rodent Model of Cardiopulmonary Disease Following Exposure to CAPs
Acute (24h post-exposure) effects of nose-only inhalation exposure to RTP CAPs on concentrations of bronchoalveolar lavage fluid (BALF) and hematologic constituents in awake, adult Sprague-Dawley rats (n=24 rats, 6 rats/group). A subset of rats was injected with monocrotaline and allowed to recover for 14 days prior to CAPs exposure. **Figure a** shows changes in concentrations of macrophages and neutrophils at 24 hr post-exposure; **Figure b** shows changes in protein and albumin in BALF obtained at 24 hr post-exposure; **Figure c** shows changes in fibrinogen and plasma ACE activity at 24 hr post-exposure; **Figure d** shows serum C-reactive protein levels at 24 hr post-exposure. All data are presented as group means ± SE, * denotes significant difference from SAL-AIR and † denotes significant difference from MCT-AIR.

CONCLUSIONS AND IMPACT

- These studies underscore the inherent complexities of conducting discrete, limited toxicology studies using environmentally-relevant exposure protocols in order to verify large scale epidemiological studies
- Furthermore, these studies emphasize the importance of companion source characterization/apportionment studies to the overall PM research effort
- Determination of small, consistent changes in cardiopulmonary functional parameters in compromised rodents may help identify specific biologically-plausible mechanism/s responsible for the adverse effects observed in epidemiological studies.

FUTURE DIRECTIONS

- To further investigate the effects and mechanisms of PM-induced toxicity using environmentally-relevant exposure scenarios, we propose to develop, characterize, and test new animal models of susceptibility and/or cardiopulmonary disease.
- These animals will be exposed to RTP CAPs and subjected to the full battery of experimental procedures available in our laboratories. Particular emphasis will be placed on developing and improving specific cardiovascular methodologies, such as electrocardiographic interval and heart rate variability analyses, for application to rodent models, as well as improved statistical techniques to better identify subtle differences in functional endpoints.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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